Light-Driven Synthesis of the Large Subunit of Fraction I Protein by Isolated Chloroplasts

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Treatment of greening cells with 70S ribosomal inhibitors such as lincomycin results in the inhibition of the synthesis of fraction I protein, but not that of other photosynthetic enzymes or chloroplast RNA polymerase (Ellis & Hartley, 1971). Fraction I protein is the major protein found in chloroplasts, and consists of large and small subunits (Rutner & Lane, 1967). The differential labelling of the large and small subunits *in vivo* (Kawashima, 1970) and the sensitivity of such labelling to chloramphenicol and cycloheximide suggests that only the large subunit is made by chloroplast ribosomes (Criddle *et al.*, 1970) However, conclusive evidence can only come from studies with isolated chloroplasts.

Isolated chloroplasts are known to carry out the incorporation of labelled amino acids into protein by means of a 70S ribosomal system, but there has been no convincing identification of any specific protein that these ribosomes synthesize *in vitro* (Kirk, 1970). We believe that the reason for this is that precautions were not taken to ensure that incorporation takes place only in intact chloroplasts in which conditions for correct termination and release of polypeptide chains are likely to be optimal. We now report that isolated intact pea chloroplasts synthesize the large subunit of fraction I protein but not the small subunit.

Chloroplasts were isolated by the rapid method of Ramirez *et al.* (1967) from 7–10-day-old pea plants (*Pisum sativum*) grown at 2000lx on a 12h photoperoid. Incorporation of [^{14}C]leucine into protein is stimulated 20-fold by red light in the absence of either added ATP or catalysts of photophosphorylation; rates of incorporation are in the range 0.5–1.0nmol of [^{14}C]leucine/h per mg of chlorophyll at 20°C. Incorporation is inhibited by chloramphenicol, by lincomycin and by lysis of the chloroplasts, but not by ribonuclease. Lysed chloroplasts supplemented with ATP and GTP show very low incorporation. We believe therefore that protein synthesis is proceeding only in intact chloroplasts.

Analysis of the chloroplast soluble protein by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and gel filtration revealed only one labelled product, which migrates exactly with the large subunit of fraction I protein. This product is not found at zero time, in chloroplasts incubated in the dark or in the presence of chloramphenicol.

Criddle, R. S., Dau, B., Kleinkopf, G. E. & Huffaker, R. C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 621
Ellis, R. J. & Hartley, M. R. (1971) *Nature (London)* **233**, 193
Kawashima, N. (1970) *Biochem. Biophys. Res. Commun.* **38**, 119
Kirk, J. T. O. (1970) *Annu. Rev. Plant Physiol.* **21**, 11
Ramirez, J. M., Del Campo, F. & Arnon, D. I. (1967) *Proc. Nat. Acad. Sci. U.S.* **59**, 606
Rutner, A. C. & Lane, M. D. (1967) *Biochem. Biophys. Res. Commun.* **28**, 531

Pathways of Malate Oxidation in Isolated Plant Mitochondria

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Plant mitochondria differ from animal mitochondria in that they will readily oxidize malate in the absence of an oxaloacetate-removing system (Palmer, 1967). Further, it has been reported that rotenone only partially inhibited this oxidation and markedly decreased the ADP/O ratio; the latter feature was not observed when succinate was used as the substrate (Ikuma & Bonner, 1967). It has been reported (Palmer & Passam, 1971; Coleman & Palmer, 1971) that plant mitochondria contain two NADH dehydrogenases localized on opposite sides of the inner membrane: the dehydrogenase on the inside of the membrane is inhibited by rotenone and oxidizes endogenous NADH, and the dehydrogenase on the outside of the membrane is inhibited by ethyleneglycol-bis-(β-amino-ethyl-ether)N,N′-tetra acetic acid, is activated by Ca^{2+} and is responsible for the oxidation of exogenous NADH.

We now report that there are also two pathways for oxidation of malate in plant mitochondria. By using ferricyanide as a non-penetrating electron acceptor and butylmalonate as an inhibitor of malate uptake it has been possible to show that they are located on opposite sides of the inner membrane. Determination of the products formed as a result of malate oxidation suggested that malate dehydrogenase was responsible for the oxidation in the inner compartment and that an NAD-linked 'malic' enzyme was responsible for the oxidation in the outer compartment. With the use of rotenone or piericidin A to inhibit the internal dehydrogenase and ethyleneglycol-bis-(β-amino-ethyl-ether)N,N′-tetra acetic acid to inhibit the external dehydrogenase it was possible to show that the malate dehydrogenase was linked to electron transport by the internal NADH dehydrogenase and coupled to phosphorylation at three sites, whereas the 'malic' enzyme utilized the external NADH dehydrogenase and was coupled to phosphorylation at only two sites. The isolation and partial purification of the 'malic' enzyme from Jerusalem-artichoke mitochondria has been carried